COMBINATIONS OF CURRENT AND ANTECEDENT CONDITIONS IN RELATION TO WING-PRODUCTION OF APHIDS[1]

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The influence of environmental conditions and genetic or other internal factors upon wing-production in the aphid *Macrosiphum solanifolii* has been repeatedly demonstrated in controlled experiments (Shull, 1928, 1929, 1932). For the most part the environmental factors have been tested singly, while all other agents were kept as nearly constant as possible. This was the best way to demonstrate that a given agent has an influence. In the course of the experiments, however, it became evident that the effect of one factor might easily depend upon the accompanying conditions. It was not merely that the factors often worked in opposite directions, so that their combined effect would be the algebraic sum of their single effects; it was found that the action of one agent might change, not merely in amount but even in sign, in response to changes in other factors.

It became desirable, therefore, to use the several agents in their various combinations. The experiments here described constitute the first exploration of the possible modifying, accentuating, and inhibiting effects. Light, temperature, and the presence or absence of wings in the parent aphids are the chief factors so far tested; and since it would be impossible with the facilities available to use all the known modifying agents, it was decided to use only these three. As a further means of curtailing the labor, only two conditions in each of these fields were employed. The two conditions chosen were such as were known to have different effects, and as would permit a not too slow accumulation of data. With respect to light, the two conditions were continuous light and alternating light and darkness (eight hours of the former, sixteen hours of the latter). As to temperature, 24° and 14° C. were selected; temperatures outside of this range are apt to have deleterious effects. The nature of the parent aphids fell into the two classes, wingless and winged, though it would have been possible and desirable to use intermediate-winged individuals as well.

To these three groups of conditions there was reason to suspect a fourth should be added. This was the set of conditions under which

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the stock of aphids was living from which the parent aphids were drawn for the experiments. While it was desirable that various sets of "stock" conditions be tested (involving light, temperature and wings), no more than one of these could be used within the limits of time and space; and temperature was selected as the condition to be varied in the stocks. Accordingly, more than three years ago one stock was started at 24° C., another at 14° C., both in continuous electric light. Later a third stock was kept at alternating temperatures, 24° during eight daytime hours, 14° for sixteen hours at night. Continuous electric light has been furnished for all these stocks, and daylight was practically excluded. One of the stocks was killed by the breakdown of the constant temperature apparatus, but was at once replaced and soon appeared to give results identical with those of the original stock.

The experiments have started with aphids from each of these three stocks. Some of the aphids from each stock were winged, others wingless. Each kind (winged and wingless) was divided into two groups, one raised at 24°, the other at 14°. At each of these temperatures, some were given continuous light, others alternating light and darkness (eight hours light, sixteen hours darkness). Twenty-four groups of parent aphids were thus necessary for a complete experiment. For some of these groups the conditions under which they were reared represented merely a continuation of their former conditions. For others, they represented a change in temperature, or in light, or in both temperature and light. Only the parents were subjected to the conditions named. Their offspring were brought, in 2-day or 4- or 5-day batches depending on temperature, to room conditions to complete their growth.

Owing to the erratic fluctuations to which wing-production in nearly all aphids seems to be subject, a single experiment of the sort just outlined could not be expected to give reliable results. The experiments have therefore been many times repeated. In this paper are recorded over 167,000 aphids. The rearing of such large numbers has been made possible only by the watchfulness and meticulous exactness of Dr. Helen F. Price; without her aid the experiments must have failed long ago. Over long periods of time the repetitions of the experiments have tended to give constant results; that is, in nearly every test there have been the same kinds of differences between the various groups of offspring. This degree of uniformity in the general results engenders confidence that the contrasts shown by the totals do actually represent the effects of the various combinations tested.

Results of the Experiments

While it would be instructive to give the results of the several repetitions of each test, or even the daily output of each group of parents, in

TABLE I

The number of winged and wingless offspring from parents derived from certain stocks and reared under certain conditions. Data are arranged to show most directly the influence of light.

Parents				Offspring		
Winged or wingless	From stock reared at temperature	Conditions under which reared		Wingless	Winged	Percentage winged
		Temp. ° C.	Light			
Wingless	24°	24	Cont.	3657	3662	50.0
			8–16	3722	1798	32.6
		14	Cont.	4021	3509	46.6
			8–16	4242	881	17.2
	14°	24	Cont.	3309	3315	50.0
			8–16	2474	2482	50.1
		14	Cont.	3207	3952	55.2
			8–16	3750	1081	22.4
	Alt.	24	Cont.	2105	2405	53.3
			8–16	2569	1954	43.2
		14	Cont.	3996	2384	37.4
			8–16	4037	667	14.2
Winged	24°	24	Cont.	4716	7002	59.6
			8–16	3678	2428	39.8
		14	Cont.	9358	7127	43.2
			8–16	12465	3208	20.5
	14°	24	Cont.	5226	2747	34.5
			8–16	5140	1422	21.7
		14	Cont.	5162	1384	21.1
			8–16	2990	439	12.8
	Alt.	24	Cont.	2585	3682	58.8
			8–16	2635	2864	52.1
		14	Cont.	4487	3131	41.1
			8–16	4134	598	12.6
Total				103665	64122	

order to show the fluctuations referred to in the preceding section, space forbids any such detailed presentation. The total numbers are large enough, it is believed, to insure that the fluctuations largely neutralize

one another. The data have not been given statistical treatment because it is uncertain what the real unit of expression is. The validity of the contrasts shown seems to be assured by the fact that in most in-

TABLE II

The same data as those given in Table I, arranged to show most directly the influence of the temperature at which the parents were reared.

Parents				Offspring		
Reared in light	Winged or wingless	From stock reared at temperature	Reared at temp.	Wingless	Winged	Percentage winged
Cont.	Wingless	24°	24°	3657	3662	50.0
			14°	4021	3509	46.6
		14°	24°	3309	3315	50.0
			14°	3207	3952	55.2
		Alt.	24°	2105	2405	53.3
			14°	3996	2384	37.4
	Winged	24°	24°	4716	7002	59.6
			14°	9358	7127	43.2
		14°	24°	5226	2747	34.5
			14°	5162	1384	21.1
		Alt.	24°	2585	3682	58.8
			14°	4487	3131	41.1
8–16 hr.	Wingless	24°	24°	3722	1798	32.6
			14°	4242	881	17.2
		14°	24°	2474	2482	50.1
			14°	3750	1081	22.4
		Alt.	24°	2569	1954	43.2
			14°	4037	667	14.2
	Winged	24°	24°	3678	2428	39.8
			14°	12465	3208	20.5
		14°	24°	5140	1422	21.7
			14°	2990	439	12.8
		Alt.	24°	2635	2864	52.1
			14°	4134	598	12.6

stances the experiments can be grouped, all or most of the experiments in one group showing the same type of contrast. These facts will be fairly apparent on inspection of the data.

The total numbers of winged and wingless offspring born of parents derived from the three stocks and reared under different conditions are shown in Table I. As there arranged, it is indicated that wingless and winged parents were taken from each of the three temperature stocks (24°, 14°, alternating); that of each group some were reared at 24°, others at 14°; and that at each temperature some were reared in continuous light, others in alternating light and darkness (eight and sixteen hours, respectively). The offspring from these twenty-four sources are given at the right, with the percentage of winged individuals among them.

Contrast of Light Conditions Under Which Parents Were Reared

The arrangement of the data after any scheme similar to that in Table I is best fitted to contrast the effect of the conditions indicated in the fourth column of the branching tree. In Table I this contrast is between continuous light and alternating light and darkness. Pair by pair the numbers to the right of this column, more particularly the percentages in the last column of the table, show the different results from these two light conditions.

In every pair of experiments except one, regardless of how they differed in other respects, more winged offspring were produced in continuous light than in alternating light and darkness. In the one exceptional pair, the third in the table, the two light treatments had practically identical effects. In one other pair (the eleventh) there would be room to question the significance of the difference if it stood alone. But with every pair excepting one showing a difference of the same sign, and most of them a difference of considerable size, there can be but one conclusion: continuous light in general favors wing-production in this strain of aphids, as against alternating light and darkness.

Contrast of Temperatures at Which Parents Were Reared

The results of the same experiments are arranged in Table II with the temperatures at which the parents were reared placed in the fourth column. This position facilitates comparison of the effects of these two temperatures, since each pair of percentages in the last column of the table shows that contrast directly for one combination of the other factors.

A glance at the right column shows that all of the pairs of percentages show differences of the same sign except one. The first two differences, which include the exceptional one and another which agrees with the majority in sign, are small and are perhaps not significant. The

rest all indicate that distinctly more winged offspring are produced at high temperature (24°) than at low (14°), no matter what other conditions are combined with it.

TABLE III

The data of Table I rearranged to show most directly the effect of wings or their absence in the parents upon wing-production in their offspring.

Parents				Offspring		
From stock reared at temperature	Reared at temp.	Reared in light	Winged or wingless	Wingless	Winged	Percentage winged
24°	24°	Cont.	Wingless	3657	3662	50.0
			Winged	4716	7002	59.6
		8–16	Wingless	3722	1798	32.6
			Winged	3678	2428	39.8
	14°	Cont.	Wingless	4021	3509	46.6
			Winged	9358	7127	43.2
		8–16	Wingless	4242	881	17.2
			Winged	12465	3208	20.5
14°	24°	Cont.	Wingless	3309	3315	50.0
			Winged	5226	2747	34.5
		8–16	Wingless	2474	2482	50.1
			Winged	5140	1422	21.7
	14°	Cont.	Wingless	3207	3952	55.2
			Winged	5162	1384	21.1
		8–16	Wingless	3750	1081	22.4
			Winged	2990	439	12.8
Alt.	24°	Cont.	Wingless	2105	2405	53.3
			Winged	2585	3682	58.8
		8–16	Wingless	2569	1954	43.2
			Winged	2635	2864	52.1
	14°	Cont.	Wingless	3996	2384	37.4
			Winged	4487	3131	41.1
		8–16	Wingless	4037	667	14.2
			Winged	4134	598	12.6

If, despite their smallness, the differences in the first two pairs are significant, the presence or absence of a *change* in the temperature may be responsible for influencing wing production. In the first pair, those

parents which came from a stock reared at 24° and were continued at 24° in the experiments produced the larger number of winged offspring. In the second pair those parents which came from a 14° stock and were continued at 14° produced the more winged offspring. It is possible that mere change from one temperature to another, whether from high to low or from low to high, reduced wing-production somewhat. In any case this could be said only of the wingless parents kept in continuous light. It is difficult to attribute any such effect to mere change in any other part of the table.

Contrast of Winged with Wingless Parents

The data of the experiments are rearranged in Table III in such a way as to show most plainly the effect of wings or their absence in the parents upon wing production in the offspring. This is done by placing the nature of the parents in the fourth column so that the pairs of percentages in the last column will show that particular contrast.

The most striking fact brought out by this arrangement is that the winged parents produced notably fewer winged offspring than did the wingless parents, provided the parents had been taken from the low temperature (14°) stock—but under no other circumstances. It made no difference in what light or temperature they were reared; if only they came from the 14° stock, the winged parents yielded the fewer winged offspring.

When the parents came from the 24° or the alternating temperature stocks, it made less difference whether they were winged or not. Indeed, it might be questioned whether wings made any difference in the offspring. Of the eight contrasts from these two stocks shown in the last column, the winged parents yielded the more winged offspring in six, and fewer winged offspring in two. One of the differences is 9.6 per cent, one 8.9 per cent, a third 7.2, the others less. While it seems likely that this preponderance of the results must indicate that in general wings in the parents favor wings in the offspring when the parents come from high or alternating temperature, the influence can only be slight and is presumably modified by some other factor.

Contrast of Temperatures from Which Parents Were Taken

By placing the temperature conditions of the three stocks in the fourth column the effects of these antecedents are most clearly shown. This is done in Table IV. In that table it is shown, so far as concerns the stocks reared at 24° and 14°, that in every instance more winged offspring were produced by parents taken from the 24° stock than by those taken from the 14° stock provided the parents chosen were winged.

The differences all seem certainly significant. But if wingless females were chosen as parents, then in general more winged offspring were produced by those coming from the 14° stock. There is one exception to this latter statement, namely, the first trio of percentages, in which the

TABLE IV

The data of Table I so arranged as to show most directly the effect of the temperature at which the parents and their ancestors were reared (prior to the beginning of experiments) upon wing-production in their offspring, with special reference to the 24° and 14° stocks.

Parents				Offspring		
Winged or wingless	Reared in light	Reared at temperature	From stock reared at temperature	Wingless	Winged	Percentage winged
Wingless	Cont.	24°	24°	3657	3662	50.0
			14°	3309	3315	50.0
			Alt.	2105	2405	53.3
		14°	24°	4021	3509	46.6
			14°	3207	3952	55.2
			Alt.	3996	2384	37.4
	8–16	24°	24°	3722	1798	32.6
			14°	2474	2482	50.1
			Alt.	2569	1954	43.2
		14°	24°	4242	881	17.2
			14°	3750	1081	22.4
			Alt.	4037	667	14.2
Winged	Cont.	24°	24°	4716	7002	59.6
			14°	5226	2747	34.5
			Alt.	2585	3682	58.8
		14°	24°	9358	7127	43.2
			14°	5162	1384	21.1
			Alt.	4487	3131	41.1
	8–16	24°	24°	3678	2428	39.8
			14°	5140	1422	21.7
			Alt.	2635	2864	52.1
		14°	24°	12465	3208	20.5
			14°	2990	439	12.8
			Alt.	4134	598	12.6

wing-production was practically identical for parents taken from both the 24° and 14° stocks.

It is difficult to see any relation between the experiments performed with parents from the alternating stock and those derived from either

of the other two stocks. In some experiments such parents produced more winged offspring than did those from either of the constant temperatures, in other experiments fewer than either, and in still others a number intermediate between those from the two constant temperatures. There appears to be no general rule stating these relations.

Distribution of Wing Production Through the Family with Respect to Age

It is important in judging the effects of different agents on wing production to know how the wings are distributed among the successive offspring of the treated parents. The experiments were so conducted as to make this information available. In all experiments the parents were removed to the stipulated conditions while in the late fourth instar or just after becoming adult. Their offspring were obtained in successive groups by changing the parents to a new plant every two days if at high temperature, or every four or five days (five in early experiments, four later) if at low temperature. Most families were practically complete in six to eight such successive groups. It is possible, therefore, to ascertain how the proportion of winged offspring changed from the beginning to the end of the family. It would again be instructive to show the families separately, but space forbids. All families derived from a common source and treated in the same way are collected into one lot, just as was done with the data so far presented.

The percentage of winged offspring from the twenty-four lots of parents is shown in the twenty-four curves of Plate I. To save tabular matter the actual numbers of individuals are not given. The total number represented by each curve may be ascertained from Table I. However, the number in each of the six to eight successive lots of offspring does not appear. In general, the early offspring were much more numerous than late ones; the last lot was sometimes so small that a percentage based on it could not be very reliable.

Effect of Age

For some of the groups of parents the conditions of temperature and light at which they were reared represented no change whatever. This is true of curves *A*, *D*, *N* and *Q*, which are darkened for ease of selection. Whatever change takes place in the percentage of winged offspring from one of these groups of parents may be looked upon as in some respect an effect of age. It is of interest to find that the change with age is of the same sort in all of them; the winged offspring become irregularly less numerous the older the parents are. The decline of the winged individuals is most rapid among the offspring of wingless parents

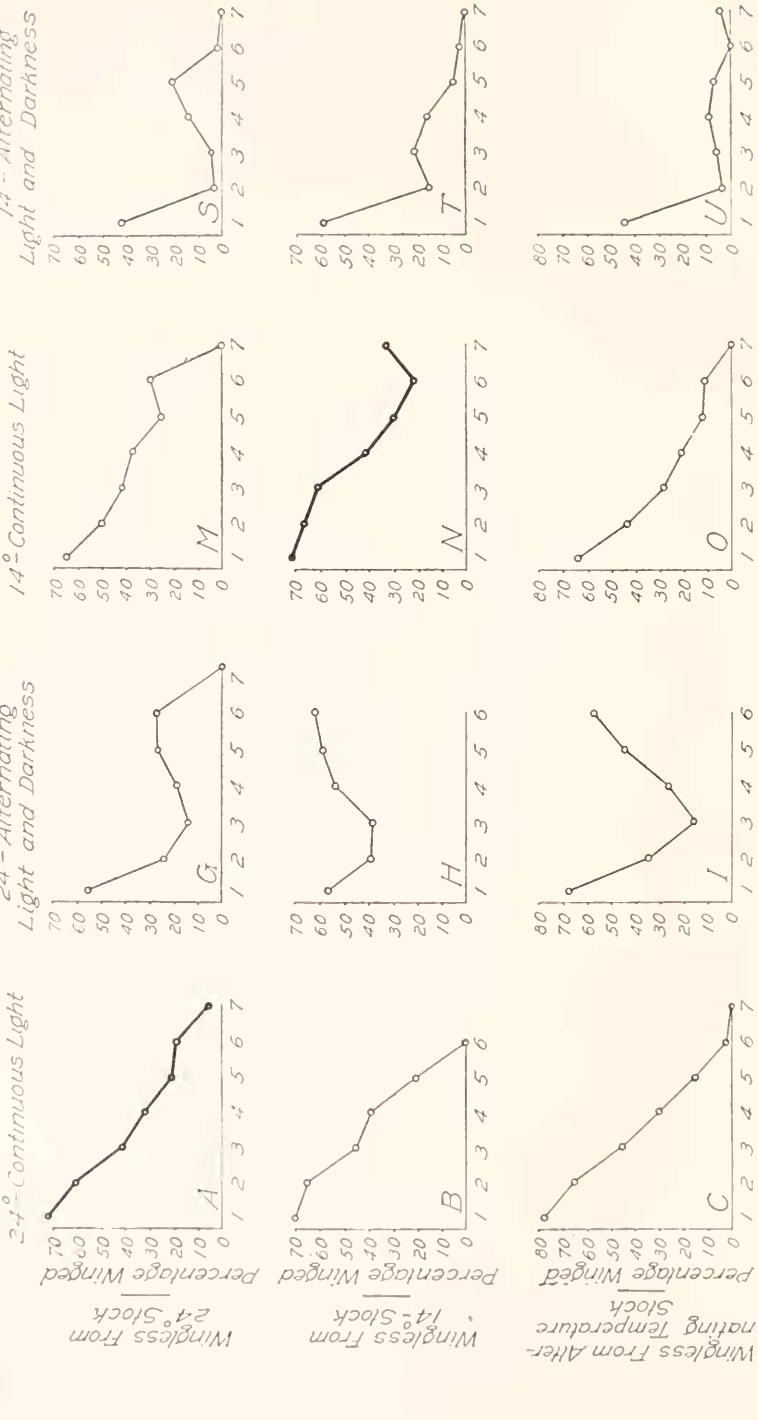
24°-Continuous Light
24°- Alternating Light and Darkness
14°-Continuous Light
14°- Alternating Light and Darkness
Wingless From 24° Stock
Percentage Winged
Wingless From 14° Stock
Percentage Winged
Wingless From Alternating Temperature Stock
Percentage Winged
A
B
C
G
H
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PLATE I

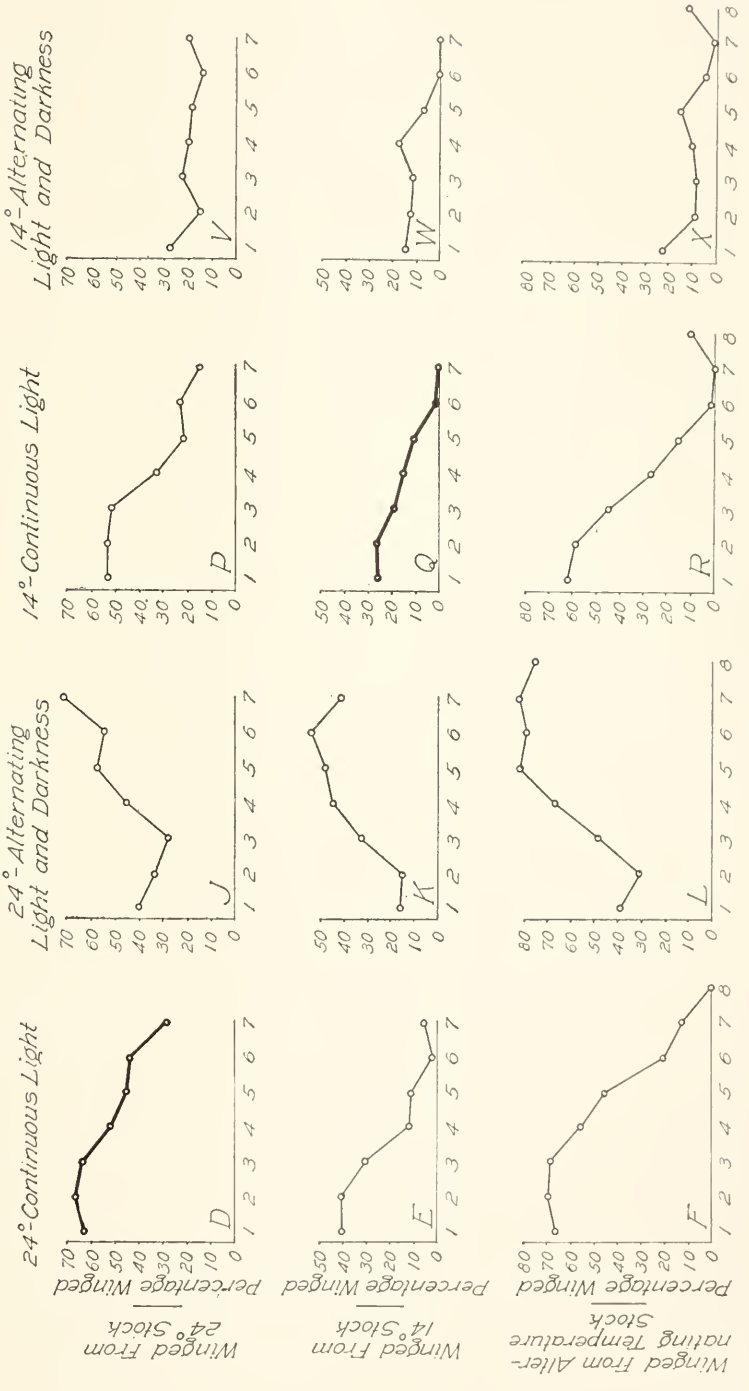

PLATE 1—*Continued*

at 24° and continuous light (curve *A*). It is slowest, but unmistakable, among those from winged parents at either 24° or 14° and continuous light (curves *D* and *Q*); however, curve *D* is throughout at a much higher level than *Q*. Whether the decline in curve *N* is greater than that in *A* may be questioned. Except at the beginning, curve *N* is everywhere higher than *A*, but it can hardly be said that the difference increases from left to right; and the sharp rise at the end of *N* is based on only thirty-three offspring in the last 4-day output.

The decline in all these curves, representing an age effect, must of course be taken into account in judging the influence of the other factors. No effect of any other factor is to be inferred unless this result of age is accentuated or reversed or partially nullified or in some way modified in at least part of the family.

Effect of Changed Conditions

All curves other than *A*, *D*, *N* and *Q* represent the distribution of winged offspring through the family as affected by one or more changes of conditions. The first column of curves (*A*—*F*) show the slightest change of any in the entire set of experiments. Curves *B* and *C* do not differ in any striking way from *A*, which means that changing the parents from 14° or alternating temperature to 24° does not modify the distribution of winged offspring as determined by mere age. With respect to winged parents, curve *E* differs from *D* chiefly in being lower; its decline is about the same as that of *E*. The low level of wing-production in *E* is not, however, due to a change of the parents from 14° to 24°, since curve *Q* is even lower. Change from 14° to 24° sufficed to raise curve *E* somewhat, but not nearly so high as *D*. This presumably means that the temperature at which the parents were reared before the experiments began influenced the amount of wing production in their offspring more than did the temperature maintained during the experiments—a statement which applies only to winged parents. Curve *F* differs from *D* only toward the end of the family, where it shows sharply less wing-production than in *D*.

The curves in the second column of Plate I have one striking feature in common, namely, a sharp dip in wing-production about the first one-fourth or one-third of the family. This dip is quite marked when the parents are wingless (curves *G*—*I*), since the initial wing-production, as in most other families from wingless parents, is high. When the parents are winged (curves *J*—*L*) the initial wing-production is quite low, and the dip is less conspicuous or may even disappear. After this depression the curve rises sharply, leveling off or even declining at the end in some. No early depression occurs in any of the curves of the

first column (*A*—*F*); in fact, there is a slight tendency in these curves for the second lot of offspring to cause a hump in the curve, either by including more winged individuals than the first lot does, or by receding less than the later curve as a whole does. These depressions must therefore be attributed to the one factor which is different in curves *G*—*L* as contrasted with *A*—*F*, namely, the alternating light and darkness to which the parents were exposed during the experiment.

Curves *M*—*R*, obtained under a single set of conditions, but from different kinds of parents whose antecedent treatment was various, are not strikingly different. They all show a decline that is mostly attributable to age. There are no constant humps nor depressions. They start high when the parents are wingless, or when the parents are winged and raised previously in alternating temperatures. Their general similarity presumably means that 14° and continuous light during the reproductive period of the parents are more influential than any change to those conditions from any antecedent conditions. The low start of curves *P* and *Q*, particularly the latter, seems to be due to the fact that the parents were winged, though winged parents previously kept in alternating temperatures (curve *R*) did not start out with a small percentage of winged offspring.

Curves *S*—*X* in the last column of Plate I are very similar. Each presents at the outset a decline which is precipitous if the starting point was high (*S*—*U*), but moderate if the percentage of winged offspring was at first low (*V*—*X*). After this decline there is a moderate rise in wing-production, the peak of which comes at various places in the family. Following the rise there is another decline.

Effect of Intermittent Light

The outstanding general result of the various sets of conditions is the effect of alternating light and darkness during the experiments. The second and fourth columns of curves (*G*—*L* and *S*—*X*) in Plate I show this effect. A reduction of wing-production in the early part of the family followed by a rise later occurs in every one of these curves. The depth of the early depression depends chiefly on the initial amount of wing-production (which in turn depends chiefly on the presence or absence of wings in the parents), while the height of the subsequent rise depends mostly on the current temperature (high temperature increasing the rise). At high temperature there is little indication of a second fall after the rise, but at low temperature such a decline is present in every instance. It may be plausibly suggested that, if reproduction continued longer at high temperature (*G*—*L*), there might be a second decline comparable with the one at low temperature (*S*—*X*).

Possible Interpretations

The results of these experiments show that every factor tested has a very noticeable effect on wing-production. A fair weighting of their effects would undoubtedly assign a greater influence to alternating light and darkness, as against continuous light, during the experiment than to any other agent. Second place would probably be taken by the temperature used (whether 24° or 14°) during the experiment. Third rating would probably go to wings or winglessness in the parents, though its effect is striking only in parents taken from a low temperature stock. And fourth place belongs to the temperature applied to the parents before the beginning of the experiment, the lowest place being assigned to this factor because it acts in a regular and marked way only on the winged parents and only in the two constant temperatures used.

The somewhat rhythmical succession of depressions and peaks of wing-production under the most influential of these agents, namely, alternating light and darkness, indicate that a moderately simple physiological explanation ought to be attainable. Such an explanation should be sought with caution, however. An attempt was made in an earlier study (Shull, 1929) to explain wing-production as due to a substance resulting from the decomposition, in darkness, of another substance produced in the light. The strain then being used for experiments was clone *A* of a later paper (Shull, 1932). As described in the latter paper, clone *A* changed radically in the fall of 1929 to become clone *A'*. It is clone *A'* that furnished the material for the experiments here reported, and wing-production in clone *A'* is in many respects different from that of *A*, even to the extent of directly reversing its response to light. A physiological explanation which fits the results from both *A* and *A'* becomes therefore difficult. It would be possible to postulate curves of physiological change of such shape that their relations to one another could be held to explain most of the facts ascertained in this group of experiments, including the rhythmical change of wing-production in curves *S*—*X* (perhaps also *G*—*L*). Until there is some known physiological feature of the aphids which corresponds to at least part of such assumptions, however, the devising of curves is of doubtful value. It seems the part of wisdom to wait.

Summary

In general, continuous light applied to the strain of aphids here used resulted in more wing-production than did alternating light and darkness, mostly regardless of the other conditions imposed.

More winged offspring were produced at high temperature than at low, in all combinations of other conditions except one. The difference in the one exceptional set of conditions was small.

A mere change of temperature from low to high or from high to low may perhaps reduce wing-production in certain of the combinations of other agents, but not in most of them.

Winged parents produced strikingly fewer winged offspring than did wingless parents if taken from the low temperature stock. Winged parents from the high temperature or alternating temperature stocks produced mostly more winged offspring than did wingless parents, but none of the differences was large.

Winged parents taken from a high temperature stock produced many more winged offspring under all other conditions than did winged parents taken from a low temperature stock.

Wingless parents generally reversed the above response, since they produced more winged offspring if taken from a low temperature stock than if taken from a high temperature, in all combinations of other conditions except one. In that one exception there was no difference between the wingless parents from the two different temperatures.

Regarding the response of parents taken from an alternating stock as compared with constant temperature stocks, no general rule can be stated. The results were very irregular.

Under uniform conditions, and without change from the conditions applied to the parents before their reproductive period begins, there is a rather rapid and steady decline in the number of winged offspring from the beginning to the end of the family. The decline is more rapid for wingless parents than for winged ones.

At high or low temperature and in continuous light the age effect described in the preceding paragraph is the chief factor governing distribution of wing-production through the family.

At high temperature and in alternating light and darkness there is a decline in wing-production early in the family, followed by a sharp rise later, regardless of the type of parents or the temperature from which they were taken.

At low temperature and in alternating light and darkness there is a decline of wing-production early in the family, a slight or moderate rise thereafter, and a decline toward the end of the family, regardless of the type of parents or the temperature from which they were taken.

The most effective of all the agents tested in these experiments is the light conditions (whether continuous or alternating) prevailing during the experiment. Temperature during the experiment is next most important. Wings or winglessness of the parents is third in importance,

followed closely by the temperature at which the parents were reared before the experiment began.

The time seems not ripe to attempt a physiological explanation which will fit these results as well as the somewhat divergent ones obtained in previous studies.

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